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VIRAL PENETRATION OF HIGH EFFICIENCY PARTICULATE AIR (HEPA) FILTERS

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14. ABSTRACT

High Efficiency Particulate Air (HEPA) filters are the primary technology used for particulate removal in many individual and collective protection applications. HEPA filters are commonly thought to be impenetrable to particulate matter, but in fact they are only 99.97% efficient at collecting the most penetrating particle (~ 0.2 micrometer). While this is an impressive collection efficiency, HEPA filters may be vulnerable to certain types of threats: Viruses are submicron in size and most have very small minimum infections doses (MID). Therefore, an appropriate viral challenge will yield penetration that exceeds the MID, for many of the threat agent viruses. Nonetheless, the overall particle size (agglomerated viruses and/or viruses attached to inert carriers) will determine the capture efficiency by HEPA filters. Aerosolized viruses are commonly thought to exist as agglomerates, which would increase the particle size and render them more prone to capture. However many of the threat agent viruses can be highly agglomerated and still exist as submicron particles. Furthermore the stability of aggregates is not well understood, and they may break apart during filtration. We have demonstrated in our laboratory that biological aerosols of MS2 coli phage, a common viral simulant, can penetrate both Carbon HEPA Aerosol Canisters (CHAC) and flat sheet HEPA material. The penetration is linear over time, thus viral penetration exceeding the MID is expected to occur in minutes following a viral challenge. We are currently investigating the particle size of the MS2 coli phage aerosol and our aim is to shift the particle size to see what effect it has on penetration. Furthermore, we are evaluating the penetration characteristics of a mammalian virus, which may better represent the threat agent viruses. This total body of work will greatly enhance our knowledge of the tactical threat posed by viral aerosols to HEPA filtration systems.

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Viral Penetration of HEPA Filters

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Abstract

High Efficiency Particulate Air (HEPA) filters are the primary technology used for particulate removal in many individual and collective protection applications. HEPA filters are commonly thought to be impenetrable to particulate matter, but in fact they are only 99.97% efficient at collecting the most penetrating particle (~ 0.2 micrometer). While this is an impressive collection efficiency, HEPA filters may be vulnerable to certain types of threats: Viruses are submicron in size and most have very small minimum infections doses (MID_{50}). Therefore, an appropriate viral challenge will yield penetration that exceeds the MID_{50} for many of the threat agent viruses. Nonetheless, the overall particle size (agglomerated viruses and/or viruses attached to inert carriers) will determine the capture efficiency by HEPA filters. Aerosolized viruses are commonly thought to exist as agglomerates, which would increase the particle size and render them more prone to capture. However many of the threat agent viruses can be highly agglomerated and still exist as submicron particles. Furthermore the stability of aggregates is not well understood, and they may break apart during filtration. We have demonstrated in our laboratory that biological aerosols of MS2 coli phage, a common viral simulant, can penetrate both Carbon HEPA Aerosol Canisters (CHAC) and flat sheet HEPA material. The penetration is linear over time, thus viral penetration exceeding the MID is expected to occur in minutes following a viral challenge. We are currently investigating the particle size of the MS2 coli phage aerosol and our aim is to shift the particle size to see what effect it has on penetration. Furthermore, we are evaluating the penetration characteristics of a mammalian virus, which may better represent the threat agent viruses. This total body of work will greatly enhance our knowledge of the tactical threat posed by viral aerosols to HEPA filtration systems.

Introduction

Biological Warfare/Terrorism is defined as deployment of biological agents to produce casualties or disease in man or animals and damage to plants or material. It is actually much further reaching than that because contamination of infrastructure, which does directly affect individuals, is a huge concern due to the extensive and costly clean up required. The potential of biological weapons was demonstrated early in world history (Hawley 2001) starting in the 14th century when plague infected soldiers were catapulted into enemy cities in an effort to spread the disease. Also, During the French and Indian war in 1754–1767, British soldiers provided American Indians with smallpox contaminated blankets and handkerchiefs. These events predate Louis Pasteur's' discovery that infectious diseases are caused by microorganisms, and clearly root biological agents as man's first attempt at creating a weapon of mass destruction (WMD). Once microorganisms were linked to human disease it did not take long for purified microbes to be used as weapons. It is well documented that many countries, including the United States, had extensive bioweapons programs (Gronvall 2005, Frischknecht 2003).

Perhaps the most feared was that of the Soviet Union. Human history is littered with many examples of microbes being deployed as acts of war and terrorism, with the most recent documented example being the attack on the Hart building in 2001. This single act of bioterrorism clearly illuminated the potential risk that biological agents pose as a weapon of terror.

Biological agents are composed four unique threats: vegetative bacterial cells, anthrax spores, viruses, and toxins. Viruses are the primary concern in this report so the others will not be discussed. The viral warfare agents are diverse and cause a variety of diseases, however their physical properties are similar (Woods 2005): They all contain a nucleic acid core surrounded by a protein coat; most also contain a lipid membrane, and are termed enveloped. Most viruses are submicron and range in size from ~25 nm – 400 nm (Hogan 2005). The minimum infections dose (MID_{50}) for all the threat agent viruses is very low as well. While absolute figures are not available, most believe that the MID_{50} are less than 10 virions (Woods). The combination of small size and low infections doses raises concern that HEPA filters may not be adequate to protect individuals from viral WMD.

HEPA filters are commonly used in individual and collective protection applications and are very efficient at removing particulate matter from the air. They are rated to be 99.97% efficient at collecting the most penetrating particle (0.3um) (Lee 1980). While this is an impressive collection efficiency, it is not absolute; 0.03% of matter at the most penetrating size does penetrate the HEPA filter. For most applications the HEPA is adequate, but toleration for viral penetration is very low, and thus only a few penetrating virions may be enough to cause disease. However, for viruses to be efficient at penetrating HEPA filters they must remain as submicron particles. Most agree that viruses will not occur as singlets when dispersed in an aerosol, rather they will agglomerate or attach to inert particle that will increase the particle sizes. It is important to note, however, that many of the threat agent viruses (*e.g.*, SARS, EEV) can be significantly agglomerated and still fall into the post penetrating range. Most of the research on bioaerosols has focused on naturally occurring biological aerosols. The research has demonstrated that a majority of particles in biological aerosols are greater than 1µm in size (Stetzenbach), and thus would not be a threat to penetrate HEPA filters. These studies, however, are all limited by the technology used to measure particles smaller than 500 nm. Therefore, the abundance of particles that would be most efficient at penetrating HEPA filters was not quantified. Studies of naturally occurring particulate aerosols (non-biological) demonstrate that nanometer size particles are actually abundant (Seinfeld 1998).

Weaponized viruses are clearly different from naturally occurring biological aerosols and the particle size for viral weapons is not clearly defined. From a weapons standpoint, it clearly would be advantageous to create smaller particles, because they would remain aerosolized longer. But in addition to creating small particles the viruses also must remain viable. The methods used to produce and protect viruses from environmental stress may dictate creating larger particles. It is unclear if weaponized viruses have been

created that are submicron in size. This uncertainty has fueled speculation that viruses may indeed be a threat to penetrate HEPA filters.

Few reports exist in the open literature on viable viral penetration studies of HEPA filters (Harstad 1967, 1969, Heimbuch 2004, Hofacre 1996). Those that do exist center on the physical performance of the HEPA, but do not evaluate the potential risk of the penetrating particles. Harstad (1969) demonstrated that viral aerosols essentially behave the same as inert aerosols and are efficiently removed by HEPA filters. Hofacre (1996) concluded the same. Both reports also demonstrate that viable viruses penetrate HEPA filters at levels that may cause disease. This conclusion however was not stated in either report. The reason for this is unclear, but it may be that 99.97% efficiency seems absolute. The reality is that 99.97% means that for every 10,000 particles that challenge the HEPA filter, three penetrate—and three virons may comprise a significant threat. The purpose of this report is to reanalyze the issues surrounding viral penetration of HEPA filters, and to shed new light on the potential for penetration. We have demonstrated in previous studies that viable MS2 coli phage can penetrate Carbon HEPA Aerosol Canisters (CHACs) (fig 1). However, it was not clear if penetration was due viruses penetrating the HEPA or was due to leaks in the canisters. In this study, the viral simulant, MS2 coli phage, was used to challenge both flat sheet HEPA material and CHACs. Both viable penetration and total penetration was measured. In addition, particle size distribution and flow velocity was varied to determine what effect each had on total and viable penetration.

Materials and Methods

Microorganisms

MS2 coli phage (ATCC 15597-B1) stock solutions were prepared by infecting 50 mL of the *Escherichia coli* host (ATCC 15597) that was grown to mid-log phase in special MS2 media (1% tryptone, 0.5% yeast extract, 1% sodium chloride, .01 M CaCl2, .002% thiamine) . The culture/infection was incubated overnight @ 37ºC/220 rpm. Lysozyme (Sigma, L6876) was added to a final concentration of 50 ug/mL, then the flask was incubated for 30 minutes at 37ºC. Chloroform (0.4%) and EDTA (.02 M) were then added and the culture was incubated for and additional 30 minutes at 37C. Cell debris was removed by centrifugation at 10,000 X g, then the supernatant was filtered thorough a 0.2 µm filter and stored at 4ºC. A plaque assay was preformed according to standard procedures to determine the MS2 titer, which typically is ~10^{11} Plaque Forming Units (PFU)/ml. For aerosol studies the MS2 coli phage was either diluted in sterile distilled water or 0.5% tryptone to a concentration ~10^{8} PFU/mL. Collison nebulizers were filled with 50ml of the solutions.

Aerosol Methods

The BioAerosol Test System (BATS, Figure 1) is a port-accessible aerosolization chamber communicating with a temperature/humidity-controlled mixing plenum and thence to a sampling plenum supplying a homogeneous aerosol to six sampling ports. Three six-jet Collison nebulizers (BGI Inc, Waltham, Mass.) deliver particles at the source that are ~2 µm mass median diameter into the mixing plenum to create the

bioaerosols. Air is drawn into a central vacuum line along a path from the sampling plenum through lines of PVC tubing (Excelon® RNT,US Plastics, Lima, Ohio). Each path runs through a test article and thence through one AGI-30 all-glass impingers (Chemglass, Vineland, N.J.) filled 20 mL of 1X phosphate buffer saline/0.001% antifoam A (Sigma, A6457). The volume of air passing in each path is controlled by a mechanical flow meter (Blue–White 400, Huntington Beach, California, or PMR1-101346, Cole–Parmer, Vernon Hills, Illinois). At the end of the sampling path, the air exhausts through a conventional HEPA filter and the vacuum pump that drives the air movement. Each sampling port is able to accommodate test articles as large as 6 inches (15 cm) in diameter.

The BATS was configured three separate ways depending on what was being tested (figure 2). In each case the total flow through each port of the BATS was set to 85 Liters per minute (LPM). The environmental conditions for all tests were ~22°C and 50% relative humidity. For flat sheet HEPA testing a portion of the flow was split off the 85 LPM flow and directed through the HEPA material (Lydall; Manchester, CT - part number 4450HS) that was compression seated and glued into swatch holders (figure 2). For CHAC tests the entire 85 LPM flow was drawn though the CHAC, but only 12.5 LPM was collected in the AGI-30 impinger (figure 2). For each test a portion of the flow was directed through a model 3936 Scanning Mobility Particle Sizer Spectrometer (SMPS) (TSI Inc, Shoreview, MN 55126) that was configured to analyze particles with a diameter of 10 nm – 415 nm. The sample flow through the SMPS was 0.6 LPM with a sheath flow rate of 6 LPM.

Viable enumeration of MS2 coli phage was determined by performing a plaque assay on the collection fluid for each AGI-30 impinger. One mL of solution from each impinger was mixed with 1ml of log phase *E. coli* grown in special MS2 media. This solution was then mixed with 9 ml of semi-solid media (special MS2 media + 1% agar) being incubated at 55°C. The total collected phage for each impinger was determined using the following formula:

$$\text{Counted PFU X Dil}^{-1} \text{ X Impinger volume}$$

Experimental Plan

Each condition tested in this study was composed of six samples that were challenged with MS2 coli phage over two days of testing: Three samples and one positive control were analyzed each day. After the filters were seated into the swatch holders they were initially leak checked by challenging with an aerosol of 100 um beads for 5 minutes. If leaks were detected the data was not used. After the leak test the BATS was loaded with MS2 coli phage and equilibrated for 15 prior to starting the challenge. The challenge was composed of four, 15 minute intervals, in which new impingers were added after each challenge. The SMPS incrementally analyzed penetration for each of the four swatch holders (3 filters and 1 positive control) for 12.5 minutes of each 15 minute challenge period.

Results

Size Distribution analysis of MS2 Aerosols in the BATS: The SMPS analysis of MS2 aerosols created in the BATS revealed that the number mean diameter was ~34 nm and the mass mean diameter was ~ 155 nm (figure 4). Both however are composed of distributions that span the entire data collection range of the SMPS. By number the amount of particles that fall into the most penetrating range for HEPA filters (100–300 nm) was only 7.5%. The curve for the mass mean diameter is not complete, but if we assume the curve is symmetrical, a reflection around the midpoint indicates that only 94% of the curve is represented by the data. The correction reveals that the amount of mass in the 100–300 nm range is 57%. Both number distribution and mass distribution of particles have been used by researchers for determining filter efficiency, but it is unclear which is more appropriate. For this analysis, the number distribution specifics a much more stringent challenge for HEPA filters than does the mass distribution.

Particulate penetration of flat sheet HEPA filters: The SMPS analysis (number and mass distributions) of the MS2 aerosols confirmed that the particle distributions and overall challenge levels for each flow rate were similar. This indicated a high degree of repeatability in the experimental set up. Penetration of particles through the HEPA filter, however, increased as flow rate increased (fig 5). This indicates the HEPA filter becomes less efficient with increasing flow rate, as expected in size regions where diffusional capture mechanisms dominate. Other researchers have reported similar observations using particles common in HEPA penetration analysis (Hofacre 1996, Lee 1990). At the low challenge concentrations at the beginning and end of the curves the penetration data disappeared into the background and thus were not meaningful. When particle penetration experiments are done for HEPA filters the particle challenge concentration is orders of magnitude greater than what can be created for biological challenges. Thus the signal-to-noise ratio is much larger. Analysis of penetration efficiency demonstrates that the most-penetrating particle (MPP) at the higher velocities is ~ 135 nm (fig 6). The lower flow rates have limited overall penetration and an MPP size can not be discriminated. The MPPs for HEPA filters are commonly believed to be 300nm, but it is actually closer to 200nm (Lee 1980). The smaller MPP observed in this study is likely due to the higher flow velocities used in our study.

Viable MS2 penetration of flat sheet HEPA filters: The viable MS2 penetration data indicate that as you increase flowrate, penetration through the HEPA also increases (fig 7); this is in perfect agreement with the SMPS data. However, the actual differences in viable penetration among the flow rates can not be directly compared due to variations in the flow-dependant AGI-30 collection efficiency. The SMPS analysis, which requires no collection, demonstrated a constant challenge for each flow rate (fig 5). If the AGI-30 had no flow-dependant variation in collection efficiency the PFU per liter of air sampled should have also been the same. An analysis of the viable MS2 challenge concentration at each flow rate demonstrated that was not the case. Instead the data indicated a positive correlation between flow rate and viable collection efficiency (table 1). This was most apparent at the 2-LPM flow rate in which no viable detection was observed over the duration of the test. A collection strategy that provided 2 cm/sec (2 LPM) through the HEPA with an additional 4 LPM of air into the impinger (fig 7) provided conditions that were efficient at capturing viable MS2 coli phage. To accurately compare the penetration

from the multiple flow rates the data were normalized: The positive control sample for each flow rate was divided by the positive control sample for the 12.5-LPM sample providing the fractional collection efficiency (table 1). The fractional collection efficiency for each flow rate was then used to normalize the penetration data. The analysis demonstrates that an 8-cm/sec velocity caused a 17fold increase in MS2 penetration compared to the 2 cm/sec velocity.

Particle penetration of CHACs: The penetration of particles through the CHAC tracked most closely with the HEPA penetration data at 2 cm/sec (fig 5). This was expected because the test flow rate of 85 L /min through the CHAC provides a face velocity of 2 cm/sec through the CHAC HEPA filter. Analysis of the penetration efficiency (fig 6) demonstrates that the CHAC penetration also follows the penetration observed for flat sheet HEPA material at flow velocities of 2 cm/sec and 4 cm/sec velocities. The overall penetration was very low and a determination of MPP was not possible.

Viable MS2 penetration of CHACs: MS2 penetration of the CHAC canister was lower than any the flat sheet HEPA material tests (fig 7 and table 1). The penetration most closely resembled that at 2 cm/sec velocity through the HEPA, as was expected due to similar face velocities. However, the total measured penetration was still 1/7 of that through the flat sheet HEPA medium. The decrease in penetration through the CHAC was likely due to the presence of the carbon bed. The carbon bed adds more surface area for the aerosol to travel through, which may be mechanically trapping the MS2 particles. However, the SMPS analysis demonstrated the particle collection efficiency of the CHAC was very similar to the collection efficiency of the HEPA at the same velocity (2 cm/sec) (fig 6). Thus other mechanisms may be responsible for the viable reduction. One possibility is that the additive ASZM-TEDA (Antimony–Silver–Zinc–Molybdenum–Triethylenediamine) in the carbon bed is exerting a biocidal effect on the bacteriophage. ASZM-TEDA is added to the carbon to prevent microbial growth and it likely has virucidal activity as well.

Particulate penetration of 0.5% tryptone nebulization solution: The addition of tryptone (0.5%) to the nebulization fluid significantly shifted the size distribution of particles to the right (fig 8). The number mean diameter size shifted to ~84nm and the mass mean diameter size shifted to ~300 nm. The percentage of particles that fall into the 100–300 nm size range, however, was only marginally changed. By number, the percentage of particles in the MPP size range increased to 38%; an increase of 30.5% over MS2 suspended in water. The mass curve is not complete, and thus the fraction of particles in the 100–300 nm size range can not be definitively calculated. However, if we assume the curve to be symmetrical the mass present in the 100–300 nm size range is 43%; a decrease of 14% over what is observed for MS2 suspended in water. The overall number of particles generated by MS2 + 0.5% tryptone and MS2 in water is not significantly different. The reason for this is that the output of particles from the Collison nebulizer is constant regardless of what is being nebulized. However, the addition of tryptone to the nebulizer caused a significant increase in the total mass being produced. Each droplet produced by the Collison contained more dissolved solids, which dramatically increased the total mass. The net result is that the MS2 coli phage was

significantly diluted in protein. The addition of the extras mass caused the HEPA filter to load with tryptone and become more efficient over time (fig 9). Filter loading was not observed for MS2 suspended in water, and penetration remained linear over time.

Viable MS2 penetration of 0.5% tryptone nebulization solution: The addition of tryptone to the nebulizer did not positively or negatively influence the viability of MS2 coliphage (fig 10): both yielded approximately the same concentration of viable MS2. However, the addition of tryptone caused a significant decrease in penetration of MS2 coliphage through the HEPA filter (fig 10). Viable MS2 penetration decreased over time and was most likely due to the tryptone loading of the HEPA filter. Although there was a shift of particles in the most penetrating range, this was overshadowed by the tremendous increase in tryptone that occluded the filter and increased the collection efficiency.

Discussion

Data presented in this report conclusively prove that viable viruses can penetrate HEPA filters. This should not be surprising given the fact that HEPA filters are rated to be only 99.97% efficient at collecting the most penetrating particle (0.2 μm). Hence, given an appropriate challenge, penetration is a mathematical certainty. The penetration is small relative to the challenge, and for most particulates this minimal penetration is not problematic. Viruses, however, pose a unique problem because very few virions are required to cause an infection (MID_{50} < 10 PFU). This problem is further exacerbated because viruses are very small (50–300 nm); individual viruses, and aggregates of viruses fall into the MPP range of HEPA filters. The data in this report were gathered from carefully controlled laboratory experiments. This approach was necessary to evaluate viable penetration efficiency of HEPA filters. However, the tactical relevance of these data is unknown because no criteria exist to determine that the BATS challenge is or is not representative of a biological attack. To determine if viral penetration of HEPA filters is a potential concern, four characteristics of viral aerosols must be considered: 1) Particle velocity (flow rate), 2) Virus concentration, 3) Duration of a biological attack, and 4) Particle size. Each of these characteristics (discussed below) will significantly impact viral penetration of HEPA filters, and ultimately determine if HEPA filters provide "complete protection" against respiratory infection by airborne viruses.

The concentration of viruses created during a biological attack is not known. The concentration will likely vary depending on distance from the distribution source. The measured concentration of viruses for this study was only 10^4–10^5 PFU per liter of air. These concentration are not excessively high and are likely lower than what would be generated during a biological attack. The duration of time that this concentration can be maintained is also an important parameter, as it directly relates to time of exposure. While there is no clear answer to this question, we do know that the penetration data observed in this study were approximately linear over time. Therefore we can predict that penetration occurs instantaneously. This may be surprising to some but HEPA filters are an "open system" that contains holes. The SMPS analysis of HEPA penetration, which was measured over the duration of the challenge confirms that particle penetration occurs instantaneously during a challenge. These data indicate that given an appropriate

challenge, an infective dose of viruses could be delivered in a matter of seconds following a challenge.

Flowrate and particle penetration are directly related. As you increase flow rate penetration will increase. HEPA filters are commonly rated for a velocity of ≤ 4 cm/sec (Hofacre 1996). Our study confirms this, demonstrating that the 4 cm/sec velocity is the cut point for obtaining HEPA performance against particle penetration. Viable MS2 coli phage penetration also increases with flow rate, with a significant increase in penetration at the higher velocities. For individual protection applications, NIOSH recommends a testing flow rate at 85 L/min; that equates to a flow velocity of 2 cm/sec for CHACs. However, breathing is more complex than simply testing at a uniform flow rate. Cyclic breathing will obviously allow penetration only during inhalation, and the most penetration will occur during peak flow velocities. Anderson et al (2006), demonstrated that maximum peak flows for average males range from 125 L/minute to 254 L/minute depending on work load (light to heavy). Peak flow were cyclic and accounted for ~ ½ the total time tested. This indicates that an average male can inhale particles at velocities greater than the rated velocities for HEPA filters.

The particle size distribution for this study was very small and may not be representative of a viral weapon attack. The challenge distribution, however, was stringent for the HEPA because only 7.5% of the number of particles fell into the most-penetrating range. In an effort to shift the particle distribution to the right, tryptone was added to the nebulization fluid. This generated more particles in the most penetrating range, but the net result was a decrease in penetration. The result is counterintuitive; however it was determined that the decrease was due to filter loading, which made the filters more efficient. This was an unexpected result, and clearly complicates the testing strategy. However, the tryptone experiment may have led to a critical observation that was not previously considered: The ratio of viable virus to inert particles may be a crucial parameter that impacts the tactical relevance of viral penetration of HEPA filters. If the ratio of virus to inert particles is small, then particle size may not be significant. The more important factor will be filter loading that will cause an increase in filter efficiency. If the ratio is large then particle size will be the dominant factor.

Summary

HEPA filters when challenged with 0.3 um particles are designed to allow penetration of .03 % of the particles. Viruses are simply particulate matter that will penetrate HEPA filters with the same efficiency as inert aerosols. This was clearly demonstrated in this study. What is not clear are the aerosol characteristics that define a viral weapons attack. Biological aerosols are complex, and many factors must be considered. The data in this report both support and refute the scenarios required for viral penetration of HEPA filters. One of the key issues that is difficult to quantify is the term “weaponization.” Can viruses be prepared so that they penetrate HEPA filters more efficiently, but still remain infectious? The answer to this question is not readily available, but it not completely unlikely. A thorough examination of past biological weapons programs may provide the answers. However, those data are hard to obtain and if available, still may not provides

clear answers. In the absence of those data, the only way to know if HEPA filters provide adequate protection is create tactically relevant biological aerosols and determine the penetration efficiency of the HEPA filters. This type of research, however, leads to a conundrum that many face in biological defense applications: The research is crucial to determine if a protection gap exists, but the research may also lead to conditions that could defeat the HEPA filter. Regardless of this question, basic research is needed to develop a better understanding of how viruses and other microbes behave in aerosols. In particular, the distribution of viruses, both viable and nonviable, among inert particles in aerosols is not well understood. Data generated from this type of research would help solve biological defense questions, but would also further basic understanding in the spread of infectious disease.

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Figure 1: MS2 challenge of CHAC ($n = 21$) in BATS
Challenge 10^3 – 10^7 PFU/Liter of air at 85 LPM

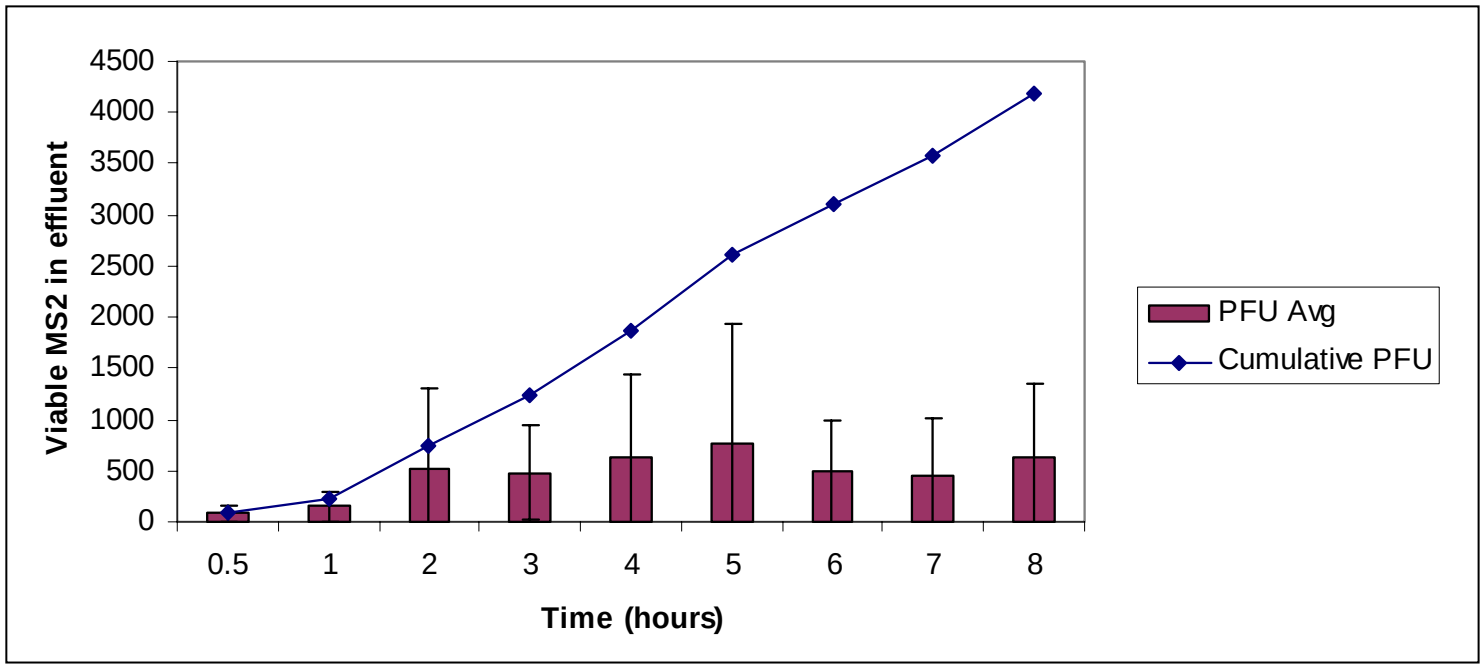

Figure 2: Biological Aerosol Test System (BATS)

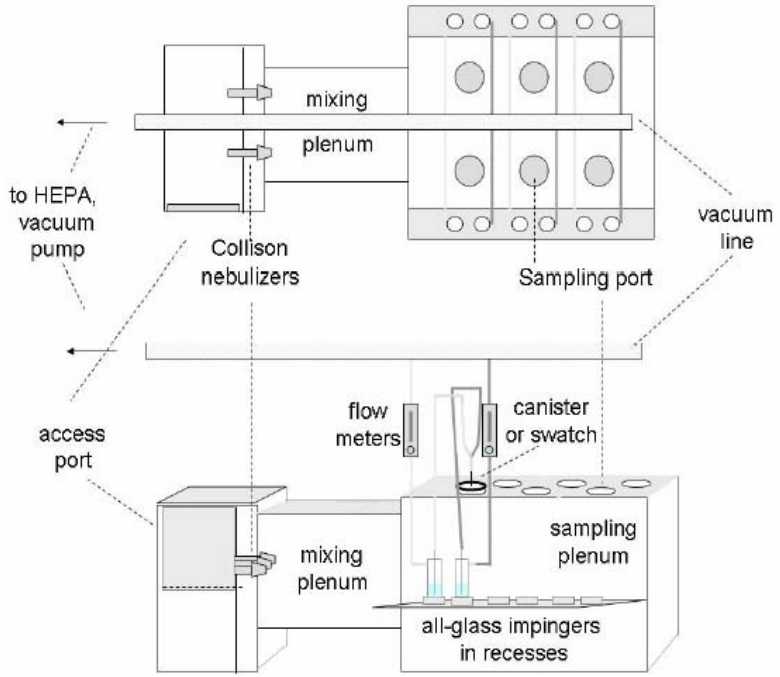

Figure 3: BATS test set up for Flat Sheet and CHAC testing

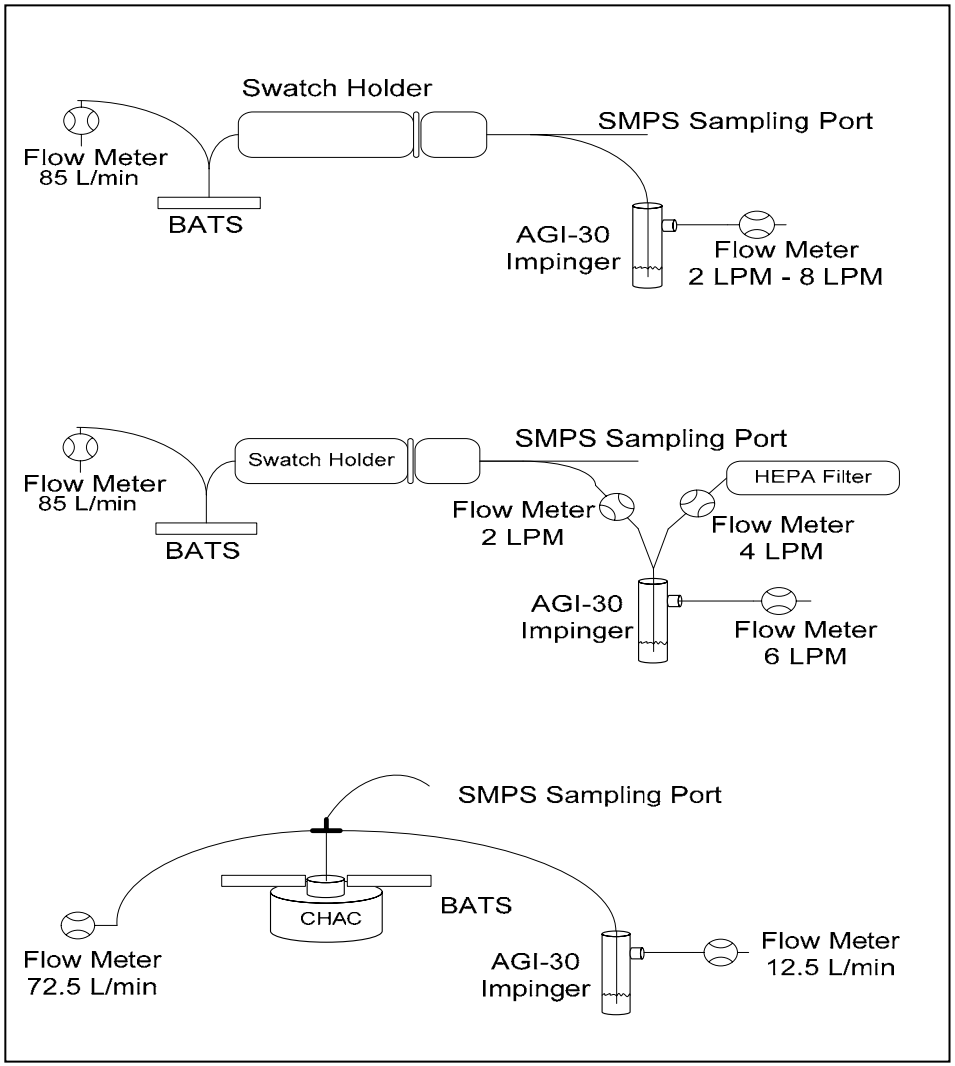

Figure 4: SMPS Analysis of MS2 Challenge in BATS

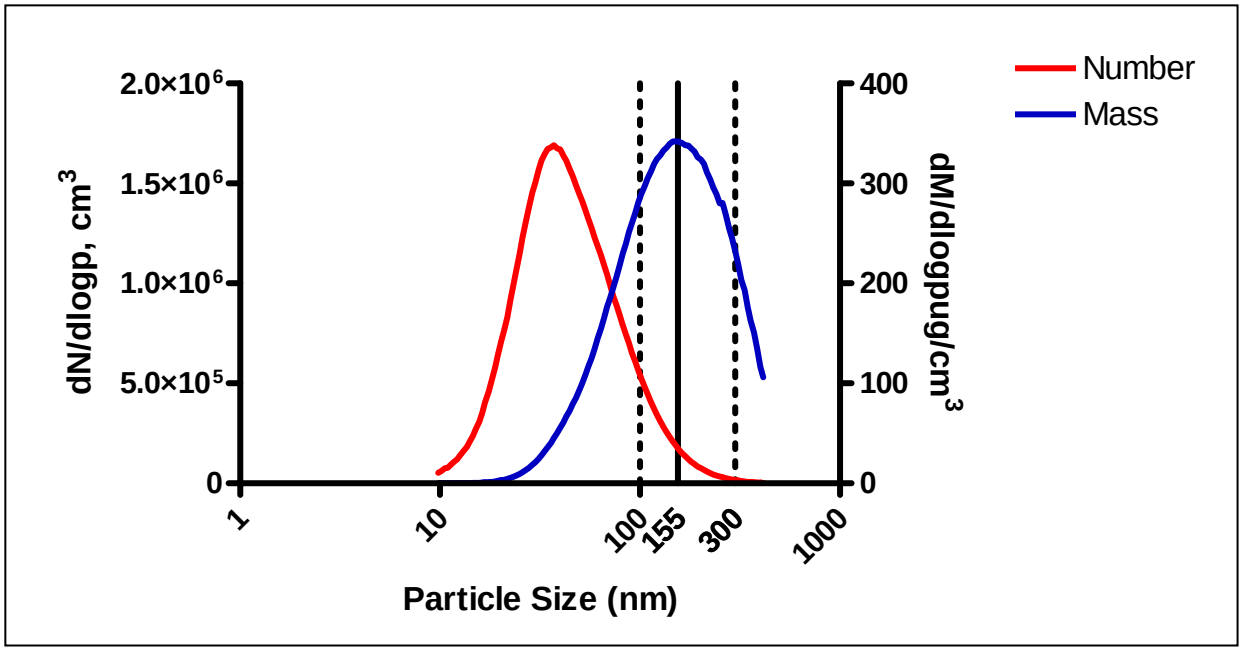

Figure 5:SMPS Analysis of MS2 Coli Phage Challenge of Flat Sheet HEPA and CHACs

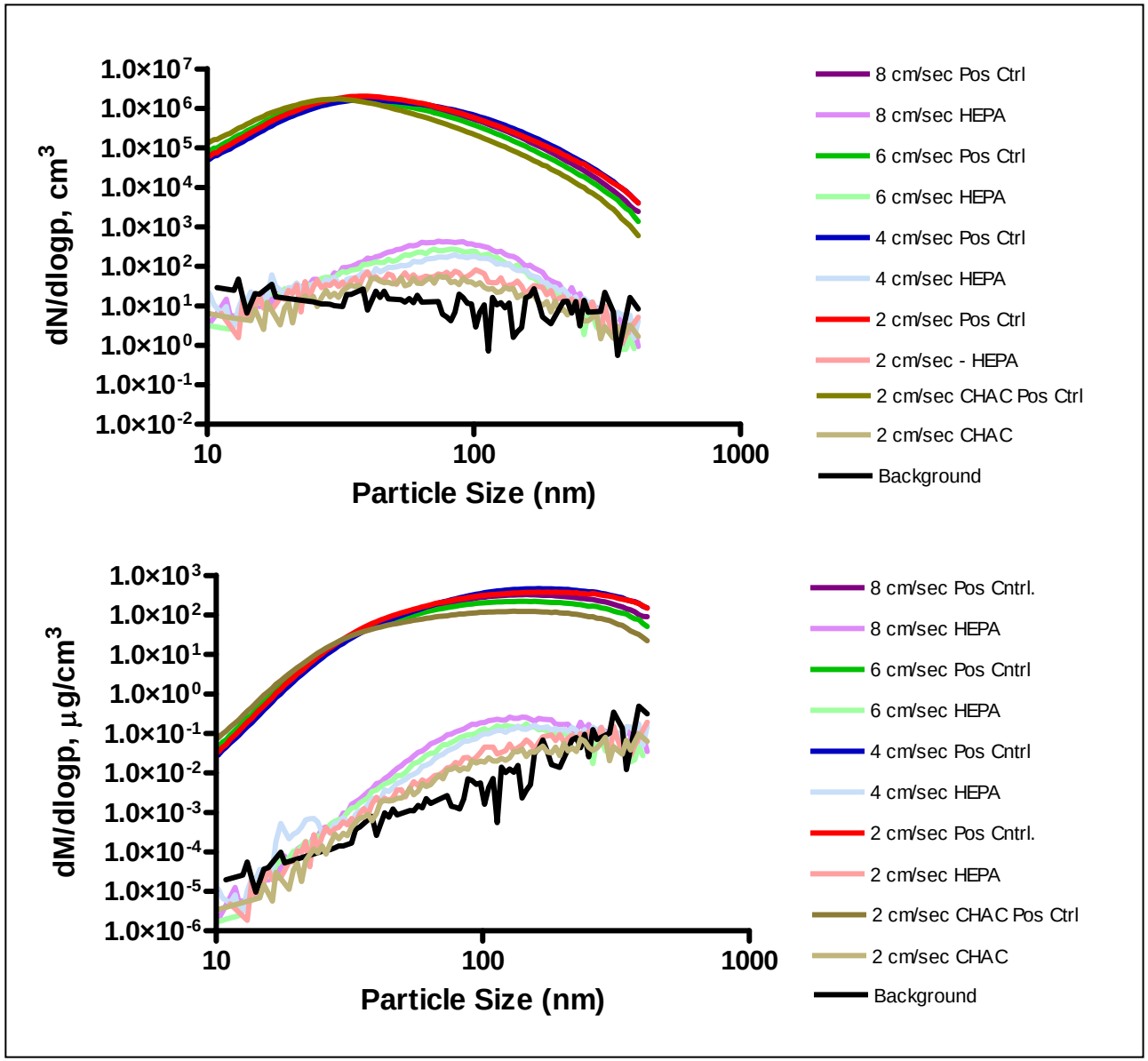

Figure 6: Filtration Efficiency of Flat Sheet HEPA Challenged with MS2 Coli Phage [(a) number , (b) mass]

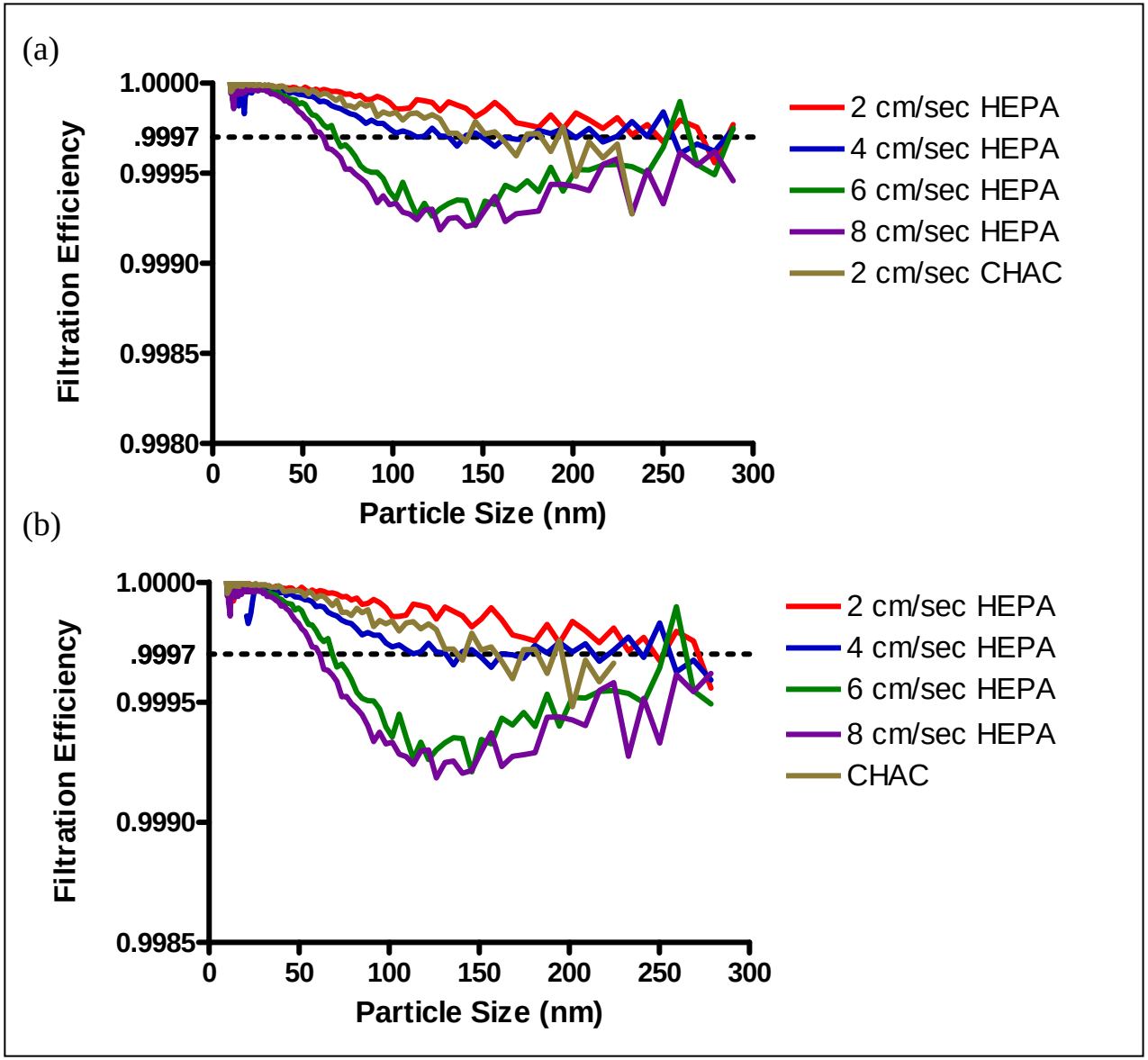

Table 1: MS2 Challenge of Flat Sheet HEPA and CHACs

Positive Controls

Condition	Collection flow rate	Measured MS2 per liter of air	Normalization factor	Normalized Positive Control
HEPA 2 cm/sec	2 LPM	1.39E+04	0.03	4.17E+05
HEPA 2 cm/sec + 4 LPM	6 LPM	8.10E+04	0.19	4.17E+05
HEPA 4 cm/sec	4 LPM	5.60E+04	0.13	4.17E+05
HEPA 6 cm/sec	6 LPM	1.42E+05	0.34	4.17E+05
HEPA 8 cm/sec	8:00 PM	1.22E+05	0.29	4.17E+05
CHAC 2 cm/sec	12.5 LPM	4.17E+05	1.00	4.17E+05

Test Samples

Condition	Collection flow rate	Measured MS2 per liter of air	Normalization factor	Normalized Penetration
2 cm/sec	2 LPM	None detected	0.03	ND
2 + 4 cm/sec	6 LPM	1.7	0.19	8.71
4 cm/sec	4 LPM	2.8	0.13	20.51
6 cm/sec	6 LPM	18.9	0.34	55.31
8 cm/sec	8:00 PM	45.7	0.29	155.88
CHAC 2 cm/sec	12.5 LPM	1.2	1	1.20

Figure 7: MS2 Challenge of Flat Sheet HEPA and CHAC — Viable enumeration

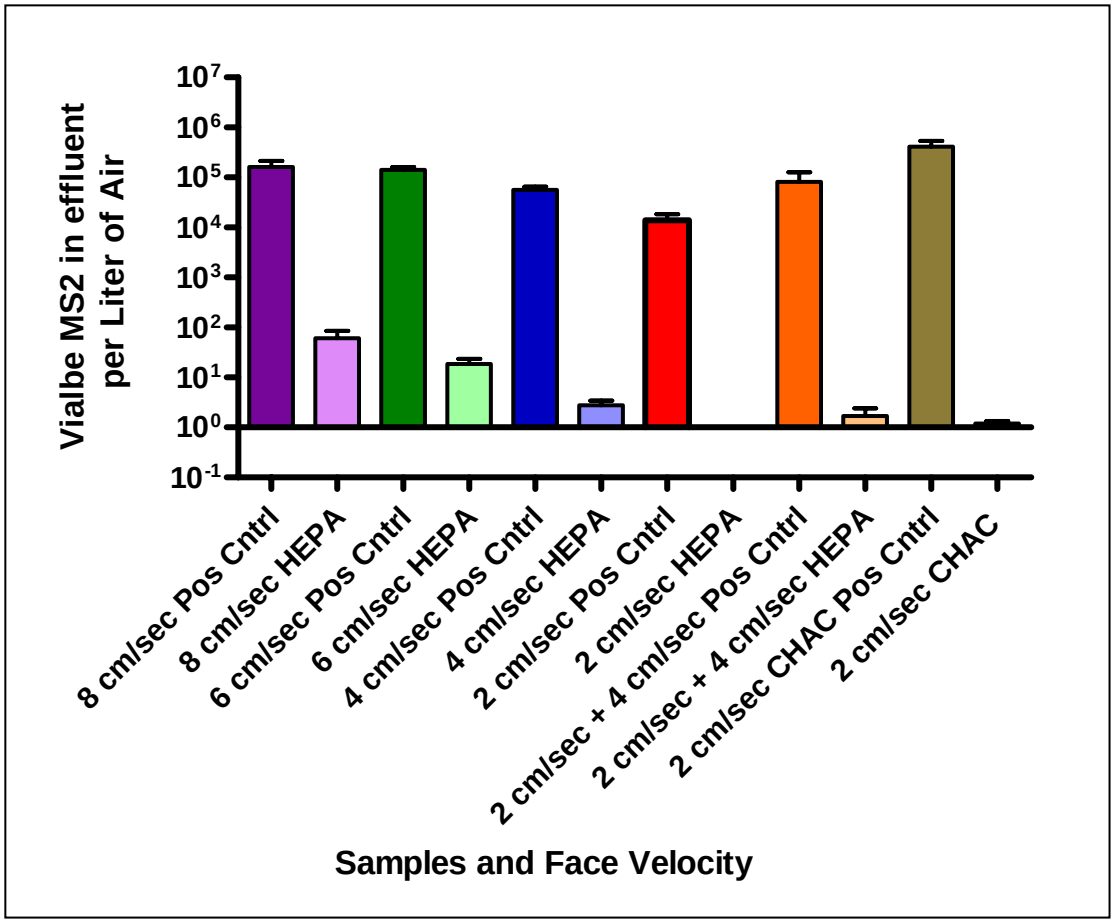

Figure 8: SMPS analysis of MS2 Coli Phage + 0.5% Tryptone in Nebulization Fluid

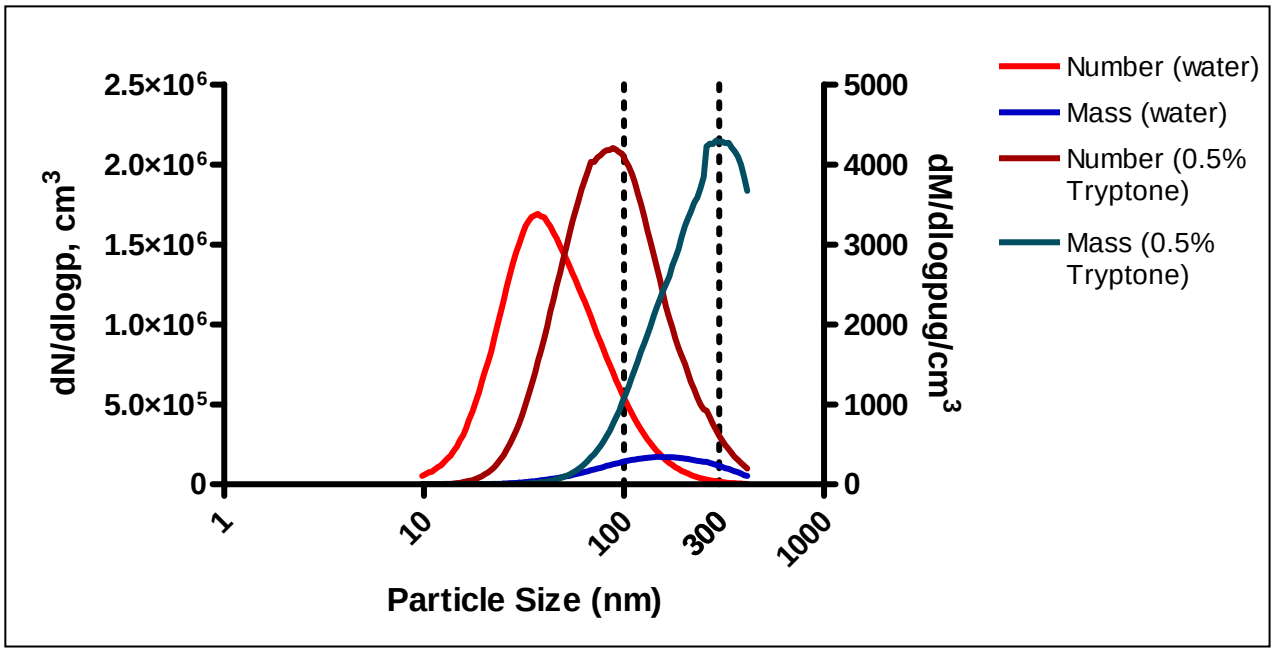

Figure 9: Filtration Efficiency of Flat Sheet HEPA Challenged with MS2 + 0.5% Tryptone

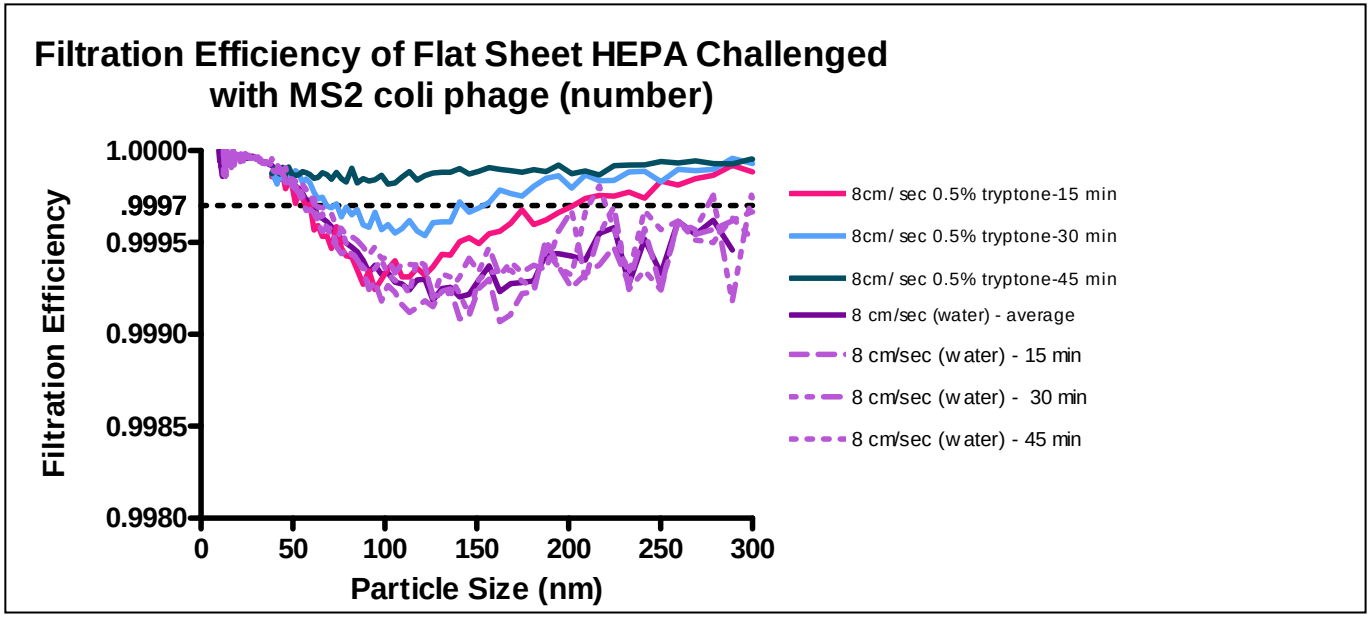

Figure 10: MS2 + 0.5% Tryptone Challenge of Flat Sheet HEPA and CHAC – Viable enumeration